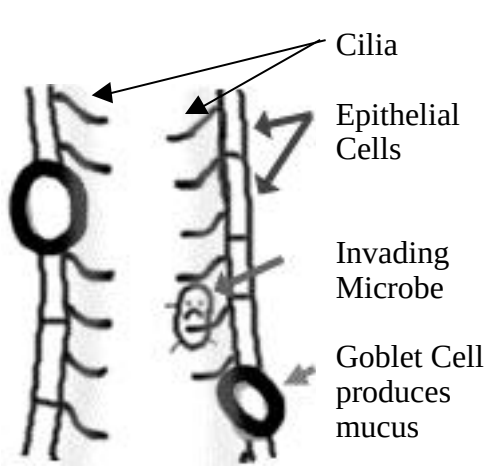
Salt Imbalance and Osmosis - Thick Mucus in Cystic Fibrosis

Introduction - In this exercise you will explore how the salt imbalance resulting from a dysfunctional chloride channel could, through osmosis, lead to the thick mucus characteristic of cystic fibrosis. This exercise will demonstrate one of the possible mechanisms by which the cystic fibrosis mutation could lead to disease. Other possible mechanisms will be discussed following the exercise.

This exercise assumes that you are familiar with cell membrane structure, ion channels, and osmosis. You can use the handout "Brief Review of Membrane Structure and Osmosis" to refresh your memory.

Respiratory Epithelium and Mucus

Epithelial Cells – are cells that line the surfaces of both are respiratory and gastrointestinal tracts. These cells serve as an interface between our body and the outside world. They serve as the surface across which we exchange nutrients and waste products.



Mucus - Mucus is a substance that coats our epithelial surfaces. It keeps surfaces lubricated and helps prevent disease by trapping potential invaders. The diagram on the left shows a section of our respiratory tract. In the respiratory tract, goblet cells secrete mucus onto the epithelial surfaces of the airway. Invading microbes can become trapped in this mucus. The epithelial cells are lined with cilia that are constantly beating and moving mucus with trapped bacteria up and out of the respiratory tract.

Mucus is a mixture of predominantly water and glycoproteins (called mucins). The mucins are produced by goblet cells that are interspersed among the respiratory epithelial cells. The composition, water content, and volume of mucus are controlled by the secretion and absorption of ions and water across the respiratory epithelium.

Cystic Fibrosis - One of the distinguishing features of cystic fibrosis is the thick mucus that lines both the airways and the intestinal tract of affected individuals. In the respiratory tract, this

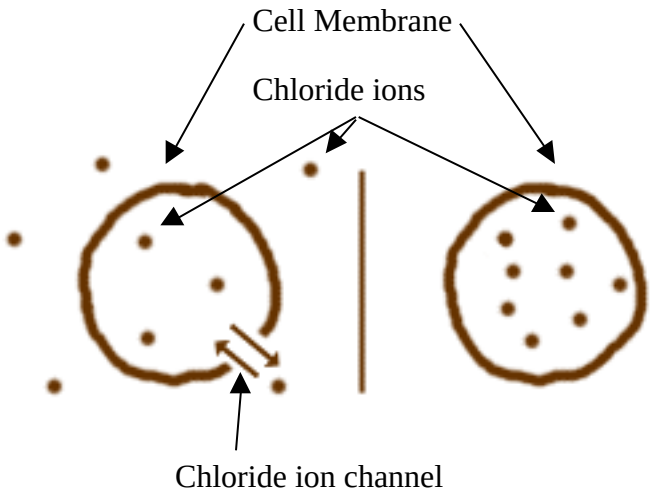
thick mucus is not removed by the cilia that usually beat and push mucus up into the throat. This thick mucus builds up in the airways, and bacteria trapped in the mucus are not removed. Thus bacterial respiratory infections are extremely common and dangerous in CF individuals. Chest pounding while the patient is in an upside down position is commonly used to help loosen and dislodge the trapped mucus.

In the gastrointestinal tract, this thick mucus clogs the ducts leaving the pancreas. Thus digestive enzymes secreted from the pancreas are unable to reach the digestive tract. This makes it difficult for CF patients to digest food and obtain enough nutrients. CF patients often take supplements of pancreatic enzymes orally to help overcome this problem.

This exercise is designed to explore how a defect in a chloride ion channel could lead to this thick mucus.

Chloride Channels in Cystic Fibrosis

In non-cystic fibrosis individuals, the affected chloride channels are found in the cells lining the epithelium of the respiratory and gastrointestinal tract. These are the same cells that are coated by the mucus described above. The chloride channels function to let chloride out of the epithelial cells. Thus, in cells from non-CF individuals, the chloride channels open periodically to allow the cell to maintain a normal balance of chloride ion between the inside and outside of the cell. In CF individuals, these chloride channels do not function, and chloride ions build up inside the cell.



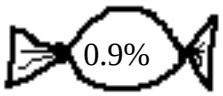
Cell from a non-CF individual. Note how chloride ions (shown as dots) can pass into and out of the cell through the chloride ion channel when it is open.

Cell from a CF individual. Note there are no chloride ion channels and the chloride ions are trapped within the cell.

In this lab, we will investigate how this absence of chloride ion channels in CF individuals might lead to their thick mucus.

Overview of the lab.
In this exercise, we will use closed dialysis bags to represent cells. The dialysis tubing itself corresponds to the cell membrane. Remember that cell membranes are semi-permeable, meaning that only some molecules can cross the membrane freely. Of particular relevance to this lab, remember that ions (Na^+ or Cl^-) are unable to cross the cell membrane freely. Special channels or transport molecules are required for these substances to cross the cell membrane. If necessary review the handout "Brief Review of Membrane Structure and Osmosis"

We will fill the "cells" or dialysis tubing, with NaCl. The normal concentration of Na^+ and Cl^- ions in the human body is 0.9%. In non-CF individuals, chloride ions will be appropriately balanced and the concentration of Cl^- both inside and outside the cell will be 0.9%. In CF individuals, the concentration of Cl^- will be much higher, since the chloride channels do not allow chloride out of the cell. For purposes of this experiment, we will assume that the Cl^- inside the cell has built up to 20%. Shown below are two "cells" with the internal concentration of Cl^- marked.



Non-CF Cell

CF Cell

After filling our cells with NaCl, we will place them in beakers containing 0.9% saline. This 0.9% saline solution represents the mucus on the surface of our respiratory tract.

Predictions
Think about what you know about membrane structure, what substances freely cross membranes, and in which direction they can move freely. Then answer the questions below.

1. If the Non-CF "cell" were placed in a beaker of 0.9% NaCl, is the concentration of NaCl higher on the inside or the outside of the cell, or equal?

Would chloride enter the cell, leave the cell, or stay where it is?

Would water enter the cell, leave the cell, or stay where it is?

2. If the cystic fibrosis "cell" were placed in a beaker of 0.9% NaCl. Is the salt concentration higher on the inside or the outside of the cell or equal?

Would chloride enter the cell, leave the cell, or stay where it is?

Would water enter the cell, leave the cell, or stay where it is?

Procedure

1. Get two 600 ml beakers, label one cystic fibrosis and the other normal. Put 400 mls 0.9% NaCl into each beaker.

2. Obtain a piece of dialysis tubing. It is important when to work carefully with dialysis tubing as it will get very small holes very easily. Tie a knot in one end. To make the knots easier to tie, twist the end about 1.5 inches and then tie this twisted portion. Fill the dialysis tubing with 0.9% NaCl (the concentration of NaCl usually found in the body). You want to add an amount of NaCl so that when tied, the bag will be full to about half capacity without a large air bubble. This will allow substances to diffuse in both directions across the membrane. When the bag is tied shut on the other end, you should be able to squeeze the bag in the middle and have the two opposite walls touch.

3. Blot any excess liquid from the outside of the bag. Weigh this cell. Note the weight in the table on the following page.

4. Put this cell into the NaCl solution in the bottom of the "normal" beaker. Every 5 min for the next 20 min, remove the bag from the beaker, blot it gently, weigh it, and then return the bag to the beaker. Note your weights in the table. While waiting, set up the second bag as described below.

5. Obtain a second piece of dialysis tubing. Tie a knot in one end. Fill the dialysis tubing with 20% NaCl following the same procedure used above.

6. Blot any excess liquid from the outside of the bag. Weigh this cell. Note the weight in the table on the following page.

7. Put this cell into the NaCl solution in the bottom of the "cystic fibrosis" beaker. Every 5 min for the next 20 min, remove the bag from the beaker, blot it gently, weigh it, and then return the bag to the beaker. Note your weights in the table.

Results -

1. Start by noting the weight of each bag before beginning the experiment, in tbe time 0 slots in the "weight of bag" column.

2. Then note the weight of each bag at 5, 10, 15, and 20 minutes. These results will go in the table on the next page in the columns labeled “weight of bag” under either 0.9% saline or 20% saline.

3. Lastly, subtract the starting weight of the bag from the weight of the bag at the time of interest.

For example – if your bag filled with 0.9% saline weighed 1.3 g before starting, and 1.5 g after 5 min, you would subtract 1.2 from 1.5 and enter 0.2 in the column labeled “weight of bag minus starting weight” across from the time labeled 5 min in the section for 0.9% as shown below. Similarly if this bag weighed 1.7 g after 10 min, you would enter 1.7-1.3 = 0.4 g in the table.

Sample table

	Contents of bag			
	0.9% NaCl		**20% NaCl**	
Time	Weight of bag (g)	Weight of bag minus starting weight (g)	Weight of bag (g)	Weight of bag minus starting weight (g)
Start (0 minutes)	1.3 g	0 g		0 g
5 minutes	1.5 g	0.2 g		
10 minutes	1.7 g	0.4 g		

	Contents of bag			
	0.9% NaCl		20% NaCl	
Time	Weight of bag (g)	Weight of bag minus starting weight (g)	Weight of bag (g)	Weight of bag minus starting weight (g)
Start (0 minutes)		0		0
5 minutes				
10 minutes				
15 minutes				
20 minutes				

Graph the results

On the graph paper below plot the change in weight over time, with time on the x-axis and change in weight on the y-axis. Draw two different lines, one for the 0.9% NaCl, one for the 20% NaCl. Be sure to label the axes, including units.

Analyze the results.
Did water move into or out of the bag? What does the difference in weight of your bag indicate about the movement of water?

How do your results compare with your predictions?

If you are unable to make a firm conclusion, discuss how you would change your experiment.

Based on your results, why might the mucus of CF patients be thicker than that of non-CF individuals?

Follow up information - Alternative Explanations
Scientists actually do not understand exactly how this chloride channel defect causes the symptoms of cystic fibrosis.

In this experiment, you explored one of the two main possible explanations that scientists are pursuing at this time (others may appear later). In the explanation that you explored, chloride is unable to leave epithelial cells and as a result, the chloride ion concentration is higher within epithelial cells which then take up water resulting in thick, dehydrated mucus. This theory is supported by the fact that patients have very thick mucus, both in the lungs, and in the digestive tract. Further, some in vitro experiments have shown increased osmosis of water into cells with a CF mutation.

The second alternative that scientists are pursuing is that NaCl concentrations are actually higher outside the respiratory epithelial cells. This high NaCl concentration may inhibit the action of antibiotics (defensins) that our respiratory tract produces, thus resulting in repeated respiratory tract infections. Evidence to support this theory includes the isolation of defensins, and showing that they are sensitive to high levels of NaCl. Also, in vitro experiments different from those described above have documented increased NaCl concentrations outside cells with a CF mutation.

Note that the two alternative explanations are contradictory to one another; one suggests higher chloride ion concentrations within CF cells, the other suggests higher chloride concentrations outside the CF cells. A major problem in addressing this question is the inability at this time to measure the NaCl concentration of the epithelial surface in a patient. Salt concentrations in expelled mucus can be measured, but vary greatly, and may not reflect the salt concentration of liquid still in a human in direct contact with the epithelial cell layer.

Further research should help sort out these conflicting results and lead to a better understanding of the molecular mechanisms underlying cystic fibrosis.